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A boronic acid-based fluorescent hydrogel for monosaccharide detection

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Abstract A boronic acid-based anthracene fluorescent probe was functionalised with an acrylamide unit to incorporate into a hydrogel system for monosaccharide detection. In solution, the fluorescent probe displayed a strong fluorescence turn-on response upon exposure to fructose, and an expected trend in apparent binding constants, as judged by a fluorescence response where D-fructose > D-galactose > D-mannose > D-glucose. The hydrogel incorporating the boronic acid monomer demonstrated the ability to detect monosaccharides by fluorescence with the same overall trend as the monomer in solution with the addition of D-fructose resulting in a 10-fold enhancement (≤ 0.25 mol/L).

1 Introduction

Monosaccharides are among the basic building blocks of life and play an essential role in the function of several physiological processes, including metabolism and cellular recognition [1]. The monosaccharide glucose serves as the main form of energy for tissues and cells [2]. Due to their biological importance, there has been extensive effort in the development of methods and techniques for monosaccharide detection [3–4].

Lorand and Edwards reported the ability of boronic acids to form complexes with 1,2- and 1,3- diols. In

addition it was discovered that D-fructose formed a 1:1 fructose-boronic acid complex and D-glucose formed a 1:2 glucose-boronic acid complex [5]. The strength of the boronic acid binding to monosaccharides is determined by the orientation and relative position of hydroxyl groups. In aqueous solution fructose predominates in the furanose form with a *syn*-periplanar pair of hydroxyl groups resulting in a strong binding constant with boronic acids [4]. As a result, a number of aryl boronic acid-based sensors have been developed for the detection of monosaccharides which exploit the difference in binding stoichiometry and inherent binding affinity to achieve either D-fructose or D-glucose selectivity [4,6–8]. More specifically, in 1994, James et al. developed an anthracene-containing mono boronic acid derivative as a photoinduced electron transfer (PET) fluorescence probe for the detection of fructose (Fig. 1) [9]. In this system, it was discovered that *ortho*-aminomethylphenylboronic acid functionality facilitated the detection of fructose in neutral aqueous solution. This pioneering work has led to the development of other *ortho*-aminomethylphenylboronic acid-containing fluorescence sensors improving selectivity, increasing excitation/emission profile and binding affinities [10–12]. While there was never any doubt that the *ortho*-aminomethylphenylboronic acid group was important to improve saccharide binding at neutral pH the mechanism of action had been under debate for a number of years [13–15]. Recently, the debate was concluded and the fluorescence enhancement on saccharide binding is caused by modulation of internal conversion resulting in different levels of quenching. Initially, before saccharide binding the free $-B(OH)_2$ groups quench the fluorescence by internal

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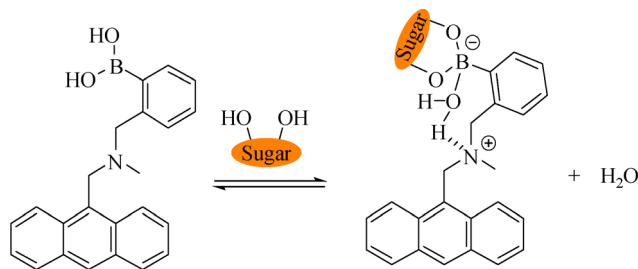


Fig. 1 Shinkai et al. anthracene-based boronic acid PET fluorescence probe for the detection of fructose

conversion, then when saccharides bind the $-B(OR)_2$ groups formed have reduced internal conversion and less quenching resulting in an enhanced fluorescence [16].

The saccharide-binding properties of aryl boronic acid derivatives have been exploited as recognition motifs across a number of different domains including polymer hydrogels [6,17,18]. Hydrogels are three dimensionally cross-linked hydrophilic polymers, with a high (~90 wt-%) water content [19]. The modification of hydrogels to contain boronic-acid binding motifs enables the physical properties of the hydrogel to be reversibly modulated through exposure to saccharide-containing stimuli, i.e., glucose responsivity [20–23]. Co-authors of this report have developed stimuli responsive hydrogels and fluorescent sensors [24–30], and as a result, we were motivated to translate a Shinkai-like anthracene-containing boronic acid sensor unit into a hydrogel sensor by linking to an acrylamide functionality, thus generating a fluorescence-on sensor hydrogel for monosaccharide detection.

2 Results and discussion

Whilst solution-based fluorescent sensors offer a significant advantage in terms of binding-kinetics over analogous heterogeneous sensors [31]. Heterogeneous immobilisation of a fluorescent sensor is preferential as it avoids contamination of the sensor in a practical situation, i.e., *in vivo* [32,33]. The near-solvated nature of a hydrogel is thus an attractive alternative as they offer heterogeneity

without the disadvantages associated with a solution-based system. By integrating the Shinkai et al. anthracene PET fluorescent probe into a hydrogel, we hoped to develop a fluorescence responsive boronic acid hydrogel, which could eliminate the need for an additional competitive optical reporter [33]. The desired boronic acid monomer **AM-5** is shown below in Fig. 2.

AM-5 was synthesised over five steps (Scheme 1). In brief, 1,6-hexanediamine was mono-Boc protected through the dropwise addition of di-*tert*-butyl dicarbonate ($(Boc)_2O$) to an excess of 1,6-hexanediamine, which afforded *tert*-butyl (6-aminohexyl)carbamate (**1**) in 74% yield. To attach the desired anthracene fluorophore, **1** was stirred with anthracene-9-carbaldehyde at room temperature overnight to form an imine intermediate. $NaBH_4$ was then added portion-wise to produce the desired secondary amine *tert*-butyl (6-((anthracen-9-ylmethyl)amino)hexyl)carbamate (**2**) in reasonable yield (48%). Compound **2** was subsequently alkylated with 2-bromomethylphenylboronic acid pinacol ester to afford **3** in good yield (89%). Compound **3** was then Boc-deprotected using trifluoroacetic acid, which also resulted in the partial hydrolysis of the boronate ester to form boronic acid **4**, this intermediate was taken onto the next step without purification. Methacryloyl chloride was then used to afford **AM-5**, which was confirmed by mass spectrometry. Compound **AM-5** proved difficult to characterise by NMR techniques and exhibited a broad and complex 1H NMR due to the formation of “oligomeric boronic acid anhydrides” [34–35].

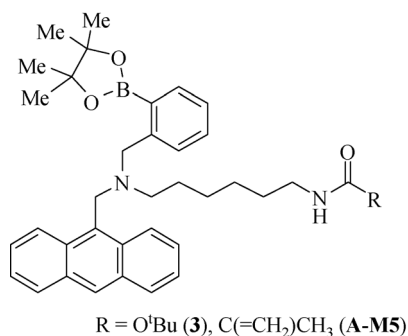


Fig. 2 Anthracene-based fluorescent monomer (**A-M5**) for the development of a hydrogel for the detection of monosaccharides

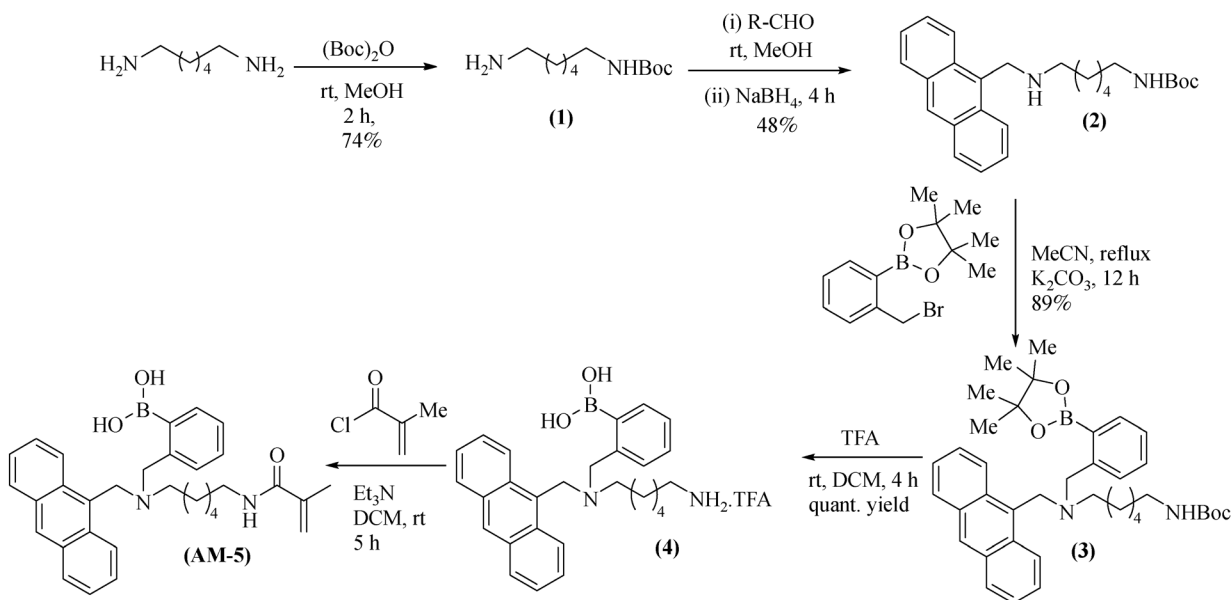
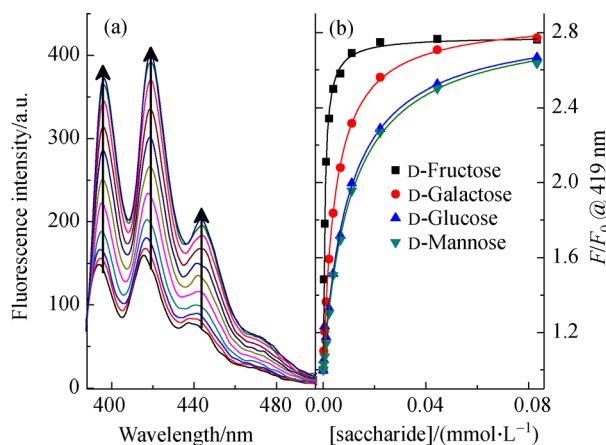
Scheme 1 Synthesis of boronic acid fluorescent probes **3** and **AM-5**

Fig. 3 (a) Fluorescent spectral changes of **3** (0.6 $\mu\text{mol/L}$) with different concentrations of D-fructose in pH 8.21 aqueous methanolic buffer solution (52.1 wt-% methanol (KCl, 10 mmol/L; KH_2PO_4 , 2.73 mmol/L and Na_2HPO_4 , 2.78 mmol/L)); (b) Fluorescence intensity changes (F/F_0) at 419 nm versus increasing saccharide concentration. $\lambda_{\text{ex}} = 370 \text{ nm}$

With **3** in hand, the fluorescence properties and responses to a panel of monosaccharides were evaluated to demonstrate its sensing ability before incorporation into a hydrogel. As shown in Fig. 3(b), **3** was more sensitive towards fructose over other monosaccharides (as expected) and the binding stability constants between mono-boronic acids and saccharides followed: D-fructose > D-galactose > D-mannose > D-glucose (Table S1, cf. Electronic Supplementary Material (ESM)). From these results, we turned our attention towards the incorporation of **3** into a hydrogel.

Hydrogels containing **AM-5** were formed by copolymerisation of acrylamide and methylene bisacryla-

mide in water through free radical polymerisation using ammonium persulfate (APS) and tetramethylethylenediamine (TMEDA) (cf. ESM for full detailed procedure). For the evaluation of the fluorescence response of the hydrogel towards different monosaccharides, each hydrogel was placed into a monosaccharide solution for 2 h (Note: 2 h was chosen since at this time point no further increase in fluorescence intensity was observed after addition of monosaccharides).

Acrylamide-based hydrogels consisting of **AM-5** were exposed to increasing concentrations of fructose and a significant fluorescence enhancement was observed (~16-fold) as shown in Fig. 4, the selectivity order for

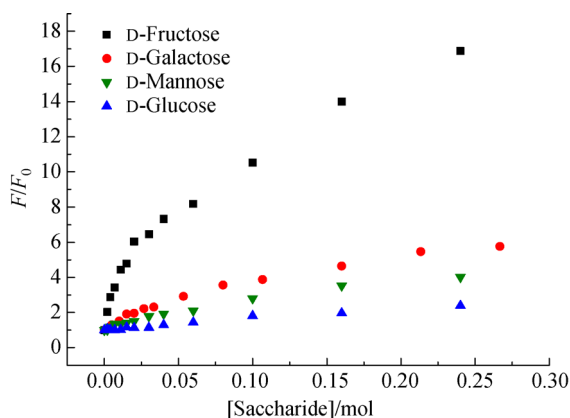


Fig. 4 Fluorescence intensity ratios of **AM-5** (F/F_0) at 409 nm versus monosaccharide concentration in a (1:1) 0.1 mol/L KH_2PO_4 /0.1 mol/L NaOH, pH 8.00 buffer solution and $\lambda_{\text{ex}} = 370$ nm

the detection of monosaccharides was consistent with the solution titration data of **3** (Fig. 2, Table S1 and Table S2), D-fructose > D-galactose > D-mannose > D-glucose. However, the observed binding constants for each monosaccharide were much lower than in the solution phase, which is believed to be due to the binding event being a diffusion-based process ((1381.7 ± 41.80) versus (52.6 ± 5.3) dm^3/mol for D-fructose). The response towards D-glucose in the hydrogel was too low for the binding constant to be determined.

3 Conclusions

A fluorescent monosaccharide responsive hydrogel was developed by functionalising the proven *ortho*-amino-methylphenylboronic acid anthracene PET sensor with an acrylamide unit to incorporate into a hydrogel backbone. The boronic acid-containing hydrogel produced a significant fluorescent enhancement (~16 fold) with the addition of fructose and the binding stability constants followed the well-established order for binding between mono-boronic acids and saccharides: D-fructose > D-galactose > D-mannose > D-glucose.

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Electronic Supplementary Material Supplementary material is available in the online version of this article at <https://doi.org/10.1007/s11705-019-1812-5> and is accessible for authorized users.

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